Reprinted from: Issues in new crops and new uses. 2007. J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA.

Potential of Annual Cereal Crops to Serve as Fuel Ethanol Feedstock and Livestock Feed

Chengci Chen*, Ratna Sharma-Shivappa, Ye Chen, Dave Wichman, and Duane Johnson

Increased public concern about global warming and over-reliance on foreign petroleum oil has led to the development of renewable and clean energy in the United States. Ethanol blended gasoline burns more efficiently and can contribute to reduction in greenhouse gasses emission (Wang et al. 1999). Ethanol production in the US has increased rapidly in recent years with a total production of 3.9 billion gallons in 2005 (RFA 2007), with maize as the major feedstock for fuel ethanol production. Since little maize grain is produced in Montana due to the cool weather and short growing season, alternative feedstocks need to be explored for fuel ethanol production.

There is an abundant but underutilized supply of agricultural residues and herbaceous grasses available in Montana. In 2006, for example, Montana produced 5.2 million tonnes (t) of wheat and 0.9 million t of barley. Over 9 million t of residues were left behind as a by-product of these crops. In addition, Montana farmers also produced 5.8 million t of hay (Montana Department of Agriculture 2006). The annual and/or perennial grasses and cereal forage crops may serve both as livestock feed and lignocellulosic feedstock for fuel ethanol production. Using winter annual triticale and sweet sorghum or pearl millet for double-cropping (two harvests per year) will allow biomass production to be further increased in the northern Great Plains. The advantage of using annual forage crops for fuel ethanol feedstock is that farmers do not need additional machinery and technologies for crop production. Although sweet sorghum and pearl millet are tropical originated plants, these crops also have potential for adaptation to temperate climate (Lueschen et al. 1991).

Due to the complex structure of the plant cell wall, pretreatment is needed to effectively convert lignocellulosics to fermentable sugars by enzymatic hydrolysis. Various pretreatment methods have been used to open up the lignocellulosic multicomponent matrix for making the carbohydrate components more accessible to hydrolytic enzymes (Lynd 1996; Wyman 1999). Based on the composition analysis of feedstocks, Chen et al. (2007b) used NaOH for triticale hay and straw, and H_2SO_4 for sweet sorghum and pearl millet hay pretreatment, respectively. The maximum xylan solubilization (78%–81%) by H_2SO_4 and maximum lignin reduction (75%–85%) by NaOH was achieved with treatment at 2.0% (w/v) at 121°C and 15 psi for 60 min. However, chemical free alternatives need to be explored to make the pretreatment process environment friendly.

Ensilage is an ancient method used by farmers to preserve forage crop for centuries (Wilkinson et al. 2003; Weinberg and Ashbell 2003). The fermentation during ensiling produces lactic acid, which results in a low pH environment to prevent degradation of carbohydrates in the feedstocks (Linden et al. 1987). In modern agriculture, a silage additive containing a combination of hemicellulase, fungal alpha-amylase, bacterial alpha-amylase, and cellulase, depending on the product and manufacture, is added to fresh forage during ensiling process to enhance fermentation and break down of plant cell wall structures (Linden et al. 1987; Henderson 1993). This process is called enzyme-assisted ensiling (ENLAC) (Schmidt et al. 1997). The acidic environment produced by ensiling also serves as a pretreatment that can result in enhanced conversion of biomass to sugars (Richard et al. 2001). Linden et al. (1987) reported that ensiling fresh sorghum results in hydrolysis of 70% cellulose to fermentable sugars. Using ENLAC process as a feedstock storage and *in-situ* pretreatment method may provide some advantages over other commonly used pretreatment methods, such as reduced energy input during pretreatment and ability to preserve and utilize fresh biomass in areas where drying of biomass feedstock is prevented by weather conditions.

* The authors acknowledge Stevenson/Basin Black Angus Feedlot for donating the land with a pivot irrigation system for this study, M&M BioTechnologies for providing SI-LO-FAME 500CS silage additive, Mohammed Moniruzzaman at Genencor International, Inc for providing Spezyme CP cellulase and Multifect xylanase, Morris Bitzer at the National Sweet Sorghum Growers Association, Anand Kumar at the Agricultural and Environmental Research Center in Canada, and Jeff Wilson at USDA-ARS for providing sweet sorghum and pearl millet cultivars for evaluation. Thanks also go to Todd Davis, Karnes Neill, and Johnna Heser for their field and laboratory technical support. Funding support came from USDA special grant to MSU Biobased Products Institute.

The objectives of this study were to: (1) evaluate the yield potentials of warm season cereal crops in the northern Great Plains; (2) develop production systems to maximize biomass yield; and (3) evaluate annual winter and summer cereal hay and straw as potential feedstocks for ethanol production and livestock feed. The sugar and ethanol per unit area produced from the various biomass and cropping systems employed in this study were estimated based on the optimal enzymatic hydrolysis and fermentation conditions reported by Chen et al. (2007a,b) following chemical pretreatment and ENLAC.

MATERIALS AND METHODS

Cultivar Evaluation for Sweet Sorghum and Pearl Millet

Four cultivars each of sweet sorghum [*Sorghum bicolor* L. Moench, Poaceae], forage pearl millet [*Pennisetum glaucum*, (L.) R. Br. Poaceae], and sorghum × sudangrass (*Sorghum bicolor*) were obtained from breeders in the United States and Canada in 2004. Sweet sorghum, pearl millet, and sorghum × sudangrass were direct-seeded into a field where the winter triticale (hybridization of *Triticum aestivum* L. with *Secale cereale* L., Poaceae) forage was mowed and sprayed with Glyphosate herbicide in central Montana, near Moccasin. The field was seeded on June 1, 2004 at a seeding rate of 23 seeds m^{-2}, using a no-till plot drill at 30 cm row spacing and a seeding depth of 1.7 cm. The experiment was a randomized complete block design with four replications. The plot dimension was 1.5 m × 7.6 m. Fertilizer of N, P, and S was applied at a rate of 112 kg ha^{-1} in the form of N–P_2O_5–K–S = 60–20–0–15 blend. Glyphosate (RoundUp Ultramax; Monsanto) was applied at a rate of 0.73 L ha^{-1} on May 14 and June 3 to control volunteer winter triticale. A tank mix of atrazine (AAtrix Nine-O; Syngenta) and dicamba (Banvel; Micro Flo) was applied on July 1 at rates of 5.6 kg ha^{-1} and 0.73 L ha^{-1}, respectively.

In 2005, 8 pearl millet, 2 sweet sorghum, and 2 sorghum × sudangrass cultivars were direct-seeded into the same field as 2004 after chopping the winter triticale/wheat (*Triticum aestivum*) mixed forage on July 7, 2005, using a no-till plot drill at a seeding rate of 45 seeds m^{-2} with a row spacing of 30 cm and with a seeding depth of 1.7 cm. The experimental design, plot dimension and fertilizer application were the same as in 2004. Glyphosate was applied at 0.88 L ha^{-1} on July 11 and a tank mix of atrazine and dicamba (Clarity; BASF, Ag) was applied on July 27 at rates of 5.6 kg ha^{-1} and 0.58 L ha^{-1}, respectively.

Biomass yield was estimated by harvesting a sample from 3 middle rows by 1.0 m length area of each plot on Sept. 9, 2004 and Sept. 13, 2005. Fresh weight of each sample was measured in the field with a portable scale, and a small sub-sample was taken and dried in an oven at 70°C for 72 hr. Samples were then ground for chemical analysis and later enzymatic hydrolysis. A separate fresh sample was taken and chopped for silage production.

Cropping Systems Development

Winter and warm season cereal crops were tested for double cropping in central Montana. For better feed quality, 50:50 mixture of winter triticale ('Triticale 102') and winter wheat ('Bigsky') seeds were solidly seeded into an irrigated field near Hobson, Montana on Sept. 20, 2004 at a seeding rate of 70 kg ha^{-1}. Fertilizers were applied following the production recommendation of 120 kg N ha^{-1} and 30 kg P_2O_5. Four plant samples were randomly cut from 3 rows by 1.0 m length area at ground level in the field once every week to determine biomass accumulation. Samples were dried in an oven at 70°C for 72 hr.

The field was split into two blocks. One block was harvested for silage at grain filling stage using a silage chopper on July 1, 2005. After chopping the winter triticale/wheat forage, sweet sorghum and pearl millet were immediately planted on July 7, 2005 using the procedures described above. Sweet sorghum and pearl millet forage were harvested on Sept. 13, 2005. Samples were collected from 3 middle rows by 1.0 m length at the ground level by hand. Samples were processed as described for 2004. The other block was allowed to grow through maturity and harvested for grain and straw on July 29, 2005. Straw and grain samples were also collected and dried.

Chemical Pretreatment and Ensilage of Feedstock

Hay samples of triticale, pearl millet, and sweet sorghum, and straw samples of triticale and wheat were ground and passed through a 1 mm sieve. Pearl millet and sweet sorghum hay samples were pretreated with H_2SO_4 at 2.0% (w/v) concentration. Triticale hay, triticale straw, and wheat straw samples were pretreated with NaOH at 2.0% (w/v). Both were autoclaved at 121°C and 15 psi for a residence time of 60 min. Detailed procedure of the chemical pretreatment are described in Chen et al. (2007b) where 2.0% (w/v) was found to be the optimal concentration. The sugar conversion of triticale/wheat straw in Table 3 was estimated based on 50:50 mixture of triticale and wheat straws. The sugar conversion of triticale/wheat hay was estimated based on triticale hay assuming triticale and wheat hay had similar sugar conversion rates (see the sugar conversion data of different hays in Table 2 of Chen et al. 2007b).

The fresh samples of triticale/wheat hay, pearl millet hay, and sweet sorghum hay were prepared for silage. A suggested rate of 10 U cellulase/g silage of SI-LO-FAME 500CS (M&M BioTechnologies, Eagle Grove, Iowa) silage additive containing 851 U/g hemicellulase, 7023 U/g fungal alpha-amylase, 8087 U/g bacterial alpha-amylase, and 1596 U/g cellulase was sprinkled and mixed thoroughly into the silage. The silage was then packed into quart canning jars. The lids of the jars were heated to aid in creating a seal. The jars were stored at room temperature for 96 to 180 days. In 2004, sweet sorghum and pearl millet fresh samples were ensiled on Sept. 22 and excavated on Jan. 26. In 2005, triticale/wheat fresh samples were ensiled on July 1, 2005, and sweet sorghum and pearl millet fresh samples were ensiled on Sept. 14, 2005. Silages were excavated on Jan. 3, 2006.

For triticale/wheat straw samples, the ground (2 mm) dry samples were hydrated to 60% moisture content, and the silage additive was sprinkled and mixed thoroughly into the silage. The silage was then packed into quart canning jars as described above (Triticale/wheat straw samples were ensiled on Aug. 31, 2005, and the silages were excavated on Jan. 3, 2006).

The silage was processed after the jars were opened. Part of the sample was placed in a large Ziploc bag to be stored in the freezer and a smaller portion was weighed and dried to determine the percent moisture and then later ground in a small cyclone grinder. One ground sample was used to determine acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) using the Ankom filter bag apparatus (Ankom Technology Corp., Fairport, New York). Cellulose and hemi-cellulose contents were estimated as cellulose content = ADF – ADL, and hemicellulose content = NDF – ADF (Schmidt et al. 1997; Jung and Lamb 2004). Cellulose and hemicellulose contents were also determined for untreated hay and straw samples.

From each jar approximately 20 g of silage was weighed and put into a 200 mL flask with 100 mL of distilled water. These flasks were then shaken on the Burrell Wrist Action Shaker Model 75 for 30 min on level three. Each sample was decanted over a funnel and screen. The filtrate was then centrifuged (Fisher Scientific Centrific Centrifuge) at approximately 4275 rpm for 30 min and the supernatant of each sample was used to measure reducing sugars and pH.

Enzymatic Hydrolysis of Chemical Pretreated and Ensiled Feedstocks

Tritical, wheat, pearl millet, and sweet sorghum feedstocks after chemical pretreatment and ensilage were prepared for enzymatic hydrolysis. Hydrolysis was carried out using Spezyme CP cellulase and Multifect xylanase (Genencor International Inc., Beloit, Wisconsin) at a ratio of 1:1.75 (v/v) of cellulase/xylanase using the optimum activity concentration of 40 FPU/g cellulose, pH 4.8, 55°C, 150 rpm for 72 hr in a shaker water bath. Detailed procedures are described in Chen et al. (2007a, b).

In 2005, sugar contents in hydrolysates of chemical pretreated feedstocks were measured by HPLC. All samples were neutralized with $Ba(OH)_2$, centrifuged at 5000×g for 10 min, and filtered through 0.22 µm Millipore filters before analysis (Chen et al. 2007b). Sugar content in this paper is reported as a sum of glucose and xylose on dry biomass basis.

In 2006, sugar contents in the hydrolysates of ensiled feedstocks were determined by measuring the reducing sugars with the 3,5-dinitrosalicylic acid (DNS) assay using the procedure described by Miller (1959). Conversion efficiency of biomass to sugars was calculated based on g reducing sugar/g biomass.

Ethanol Fermentation

Saccharomyces cerevisiae (ATCC24859) used for hydrolysate fermentation was obtained from the Microbiological Engineering Laboratory in the Department of Agricultural and Biological Engineering at Pennsylvania State University. Hydrolysates from enzymatic hydrolysis were centrifuged at 5000×g for 10 min. Twenty ml of supernatant was transferred to 100 mL serum bottles and pH was adjusted to 7.0 by adding 2 N NaOH prior to inoculation with *S. cerevisiae* at a cell concentration of 10 g dry matter/L. All samples were incubated in air-tight serum bottles at 30°C for 72 hr and analyzed for ethanol content at the end of the fermentation (Chen et al. 2007a,b).

For comparison, grains were also hydrolyzed using thermostable α-amylase produced by *Bacillus licheniformis*, and amyloglucosidase produced by *Aspergillus niger* (Sigma-Aldrich Chemical Co., St. Louis, Missouri). Saccharification of flours was carried out in 50 mL glass centrifuge tubes. One gram each (dry basis) triticale and wheat flour was mixed with 30 mL phosphate buffer (pH 6.9, 50 mM). To each flour sample, 186 μL (300 units/g flour) of thermostable α-amylase was added. The slurry samples were then stirred and incubated at 90°C for 2 hr on a shaker water bath (150 rpm). After incubation, samples were allowed to cool to 25°C. The pH of the solution was adjusted to 4.5 by adding 2 N NaOH. Each flour sample was then loaded with 200 U/g flour of amyloglucosidase. The samples were incubated at 55°C for 20 hr. Aliquots of 0.5 mL were taken at the termination of hydrolysis to determine reducing sugar content. After centrifugation of the hydrolysates at 5000×g for 10 min, twenty mL of supernatant was transferred to 100 mL serum bottles. The hydrolysate was then adjusted to pH 7 by adding 2 N NaOH and inoculated with *S. cerevisiae* to a cell concentration of 10 g dry matter/L (Palmarola-Adrados et al. 2005). All samples were then incubated at 30°C for 72 hr.

Ethanol content in the fermentation broth was analyzed by an enzymatic assay (Chinn et al. 2006). All samples were centrifuged at 5000×g for 10 min before analysis.

Fiber Analysis and Feed Quality Measurements

The biomass feedstocks of hay and straw, both untreated and ensiled were sent to the Midwest Laboratories (Omaha, Nebraska) to test feed qualities. The parameters measured include crude protein (CP), ADF, NDF, total digestable nutrients (TDN), relative feed value (RFV) and nitrate content (NO_3).

RESULTS

Biomass Yield of Sweet Sorghum and Pearl Millet

The yields of above ground biomass ranged from 6390 to 7700 kg ha^{-1} for pearl millet, 7090 to 8690 kg ha^{-1} for sweet sorghum, and 5920 to 10720 kg ha^{-1} for sorghum × sudangrass in 2004. The top yielding cultivars were 'Hybrid 192', 'Fremont', and '341 × 28' for pearl millet, sweet sorghum, and sorghum × sudangrass, respectively (Table 1). In 2005, the yield range was from 2680 to 6590 kg ha^{-1} for pearl millet, 2260 to 3700 kg ha^{-1} for sweet sorghum, and 2760 to 5650 kg ha^{-1} for sorghum × sudangrass. Due to the delayed seeding date of July 7, 2005, sorghum and millet received less heat during the growing season and therefore, produced less biomass compared to 2004 (Table 1).

Biomass and Grain Yield of Single and Double Cropping Systems

Winter triticale/wheat biomass accumulation followed a typical growth curve (Fig. 1), and the yield reached maximum by June 30, 2005, then slightly decreased during the grain ripening process. Farmers usually cut winter cereal forages at heading to grain filling stage for good feed quality, which is around June 15 in central Montana.

Sorghum and millet were harvested on Sept. 9 and Sept. 13 in 2004 and 2005, respectively. By analyzing the 96 years long-term weather data from the Central Agricultural Research Center at Moccasin, Montana, it was observed that the mean GDD (growing-degree-days with a base temperature of 10°C) between June 15 and Sept. 30 was 730 (Fig. 2), which is 91 and 139 GDD more than 2004 and 2005 growing season. Therefore, it is feasible to harvest winter triticale on June 15 and have sweet sorghum or pear millet growing from June 15 to Sept. 30. The sweet sorghum and pearl millet are expected to receive a yield equivalent to the yields in 2004

Table 1. Biomass yields of different species of warm season cereal crops at central Montana in 2004 and 2005.

Species	Cultivar	Biomass (kg ha^{-1})	Species	Cultivar	Biomass (kg ha^{-1})
2004			**2005**		
Pearl millet	Hybrid 68	6590 ± 1210[z]	Pearl millet	FPMH310	5510 ± 700
	Hybrid 76	6390 ± 1030		FPMH311	5580 ± 1270
	Hybrid 192	7700 ± 920		FPMH312	6590 ± 1390
	Hybrid 195	7280 ± 1750		PMSSH12	4480 ± 1700
Sweet sorghum	Fremont	8690 ± 1830		PMSSH504	1970 ± 820
	Simon	7090 ± 1010		PMSSH7	5240 ± 1340
	FS-5	8630 ± 1520		TifGrain 10	4300 ± 1920
	Rio	7450 ± 1400		TifLeaf 3	2680 ± 640
Sorghum × sudan-grass	327 × 23	6200 ± 540	Sweet sorghum	Fremont	2260 ± 1190
	327 × 27	6460 ± 780		Simon	3700 ± 970
	341 × 28	10720 ± 6590	Sorghum × sudan-grass	Selection 1	2760 ± 970
	372 × 36	5920 ± 1390		Selection 2	5650 ± 870
LSD		2928			2108
Growing period		6/1/04–9/9/04			7/7/05–9/13/05
Growing degree days (T_b = 10°C)		639			591

[z]Values are mean yields ± SD.

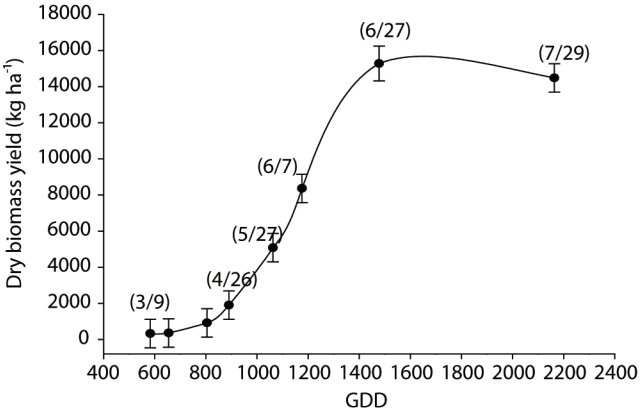

Fig. 1. Biomass accumulation of winter triticale/wheat mixture at central Montana in 2004–2005 crop year. GDD is the accumulated growing-degree-days from the date of planting with a base temperature of 10°C.

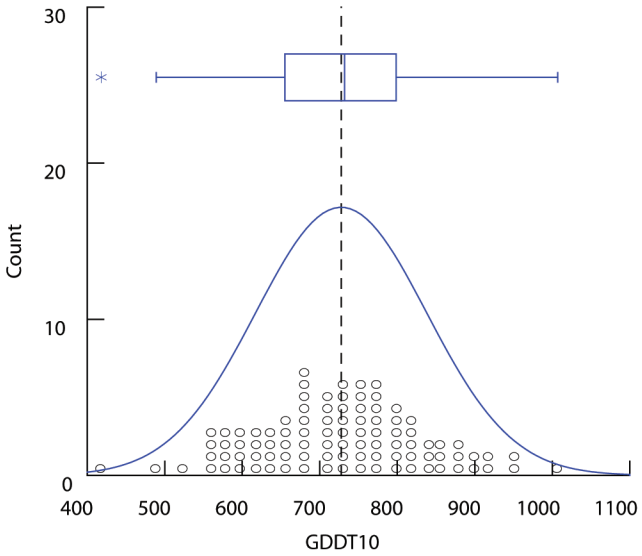

Fig. 2. Histogram of the growing-degree-days (GDD) between June 15 to Sept. 30 with a base temperature of 10°C. The diagram was generated from 96 years of long-term weather data at Moccasin, Montana.

season, assuming no other water and fertility restrictions. Based on the above assumption, biomass yields of double cropping systems with winter triticale/wheat and sweet sorghum or millet were estimated in Table 2.

Table 2. Total biomass yield for single cropping with winter triticale/wheat mixture and double cropping with winter triticale/wheat – sweet sorghum or pearl millet.

Crop	Single cropping winter triticale/ wheat (kg ha^{-1})	Double cropping (kg ha^{-1})	
		winter triticale/wheat – pearl millet	winter triticale/wheat – sweet sorghum
Winter crop straw/hay	11,350 ± 860	14,850 ± 600	14,850 ± 600
Winter crop grain	3,120 ± 670	0	0
Summer crop hay	0	7,700 ± 920	8,690 ± 1,830
Total biomass	14,470b[z]	22,550a	23,540a

[z]Values followed by the same letter in the same row are not significant different according to LSD multiple range test at P=0.05.

Table 3. Chemical compositions of hay and straw and sugar conversion by enzymatic hydrolysis after chemical pretreatment.

Species	Composition (%)				
	Cellulose	Hemi-cellulose	ADL	Sugars after hydrolysis[z]	Sugars in the check
Pearl millet hay	28.5 ± 1.3bc[y]	26.2 ± 1.5a	4.4 ± 0.5b	28.0 ± 1.2bc	0.0 ± 0.0a
Sweet sorghum hay	26.7 ± 1.4c	23.6 ± 2.2ab	4.3 ± 0.6b	24.9 ± 2.0c	0.0 ± 0.0a
Triticale/wheat hay	32.3 ± 0.2b	18.9 ± 0.1b	6.5 ± 0.2b	35.1 ± 1.6a	0.6 ± 0.0a
Triticale/wheat straw	42.9 ± 0.6a	20.7 ± 1.1b	10.1 ± 0.0a	36.7 ± 2.4a	0.3 ± 0.1a

[z]Pearl millet and sweet sorghum hays were pretreated with 2% H_2SO_4, and triticale hay and straw were pretreated with 2% NaOH. Sugars were measured by HPLC method and calculated on dry mass basis. The total sugars equal to glucose plus xylose.

[y]Values followed by the same letter in the same column are not significant different according to LSD multiple range test at P=0.05.

Table 4. Chemical compositions of hay and straw and sugar conversion by enzymatic hydrolysis after enzyme-added ensilage.

Species	Composition (%)				
	Cellulose	Hemi-cellulose	ADL	Sugars after hydrolysis[z]	Sugars in the check
Pearl millet hay	29.4 ± 1.6b[y]	15.8 ± 1.5bc	4.7 ± 0.6c	21.4 ± 3.7b	7.9 ± 2.1ab
Sweet sorghum hay	25.5 ± 3.2 c	13.7 ± 1.9c	3.9 ± 0.8c	27.6 ± 3.5ab	10.4 ± 1.9ab
Triticale/wheat hay	31.7 ± 1.0b	18.1 ± 1.2ab	8.2 ± 1.2ab	29.8 ± 3.9a	12.4 ± 3.7a
Triticale/wheat straw	39.4 ± 3.2a	21.5 ± 1.6a	11.5 ± 3.3a	29.9 ± 0.6a	0.2 ± 0.1c

[z]Sugar content was measured by DNS assay and calculated on dry mass basis.

[y]Values followed by the same letter in the same column are not significant different according to LSD multiple range test at P=0.05.

Conversion of Biomass to Sugars

Triticale and wheat straw harvested at maturity had greater cellulose and ADL contents than hay harvested prematurely (Table 3), but pearl millet hay tended to have greater hemicellulose content than triticale/wheat hay and straw. Enzymatic hydrolysis using combined enzymes of Spezyme CP cellulase and Multifect xylanase resulted in 24.9% to 36.7% sugar conversion (glucose + xylose) for the chemical pretreated feedstocks, and triticale and wheat hay and straw had greater sugar conversion than pearl millet and sweet sorghum hay.

Cellulose, hemicellulose, and ADL contents of ENLAC feedstocks were greater for triticale/wheat straw than for pearl millet and sweet sorghum hay. Triticale/wheat hay and straw had greater sugar conversion (0.30 g reducing sugars/g dry mass) than pearl millet hay (0.21 g reducing sugars/g dry mass). There were 18% and 14% of soluble sugars in the triticale/wheat and sweet sorghum hays at harvesting, and considerable amount of the sugars were preserved or produced during ensiling (see sugar contents in the check column in Table 4). Total sugar conversion rate was less after ENLAC (Table 4) than after chemical pretreatment (Table 3) especially for triticale/wheat straw.

Sugar and Ethanol Yield for Different Cropping System Scenarios

Total sugar yields per hectare were estimated (Table 5) based on the biomass yields in Table 2 and the sugar conversion rates in Table 3 and 4. The sugar conversion of grain samples was based on amylase hydrolysis of starch (0.66 g reducing sugars/g flour for triticale and wheat flour). Similar to the sugar conversion rates in Table 3 and 4, the total sugar yields were greater using the chemical pretreatment than using ENLAC. The single cropping system with winter triticale/wheat, double cropping system with winter triticale/wheat and pearl millet, and double cropping system with triticale/wheat and sweet sorghum produced 6240, 7370, and 7370 kg ha^{-1} sugars for chemical pretreated feedstocks, and 5460, 6080, and 6830 kg ha^{-1} sugars for ENLAC feedstocks, respectively.

The ethanol yield after fermentation of hydrolysates from different feedstocks with chemical pretreatment using *S. cerevisiae* ranged from 0.27 to 0.34 g/g glucose, and the ethanol yield from the ensiled feedstocks ranged from 0.21 to 0.28 g/g reducing sugars (Chen et al. 2007a,b). Based on these conversion rates, ethanol yields per hectare were estimated for the three cropping systems (Table 5). The ethanol yields were similar using chemical pretreatment and ENLAC for hays, but chemical pretreated straw feedstock produced more ethanol than ENLAC straw feedstock. This is likely due to ineffective sugar conversion of ensiled straws (Chen et al. 2007a). Among the three cropping systems, ethanol yield was 2280 to 2540 L ha^{-1} following chemical pretreatment, with little difference among the cropping systems. For ENLAC, however, the double cropping systems with winter triticale/wheat and sweet sorghum produced more ethanol (2420 L ha^{-1}) than the single cropping system (1970 L ha^{-1}). Although chemical pretreated feedstocks in the double cropping systems produced more total sugars than the ensiled feedstocks, the ethanol yields were similar between the two pretreatment methods. This is likely due to the inefficient fermentation of xylose in the chemical pretreated feedstocks with *S. cerevisiae*.

Table 5. Sugar and ethanol yields from each crop component of different cropping systems based on optimal pretreatment, enzymatic hydrolysis, and *S. cerevisiae* fermentation.

	Sugar yield (kg ha^{-1})		Ethanol yield (L ha^{-1})	
Cropping system	H_2SO_4 or NaOH	Enzyme-assisted ensilage	H_2SO_4 or NaOH	Enzyme-assisted ensilage
Single cropping:				
Winter triticale/wheat grain	2070 ± 450[z]	2070 ± 450	1070 ± 230	1070 ± 230
Winter triticale/wheat straw	4170 ± 320	3390 ± 260	1470 ± 110	900 ± 70
Sub total	6240	5460	2540	1970
Double cropping A:				
Winter triticale/wheat hay	5210 ± 210	4430 ± 180	1630 ± 70	1570 ± 60
Pearl millet hay	2160 ± 260	1650 ± 200	650 ± 80	590 ± 70
Sub total	7370	6080	2280	2160
Double cropping B:				
Winter triticale/wheat hay	5210 ± 210	4430 ± 180	1630 ± 70	1570 ± 60
Sweet sorghum hay	2160 ± 460	2400 ± 510	770 ± 160	850 ± 180
Sub total	7370	6830	2400	2420

[z]Grain samples were heat-treated and hydrolyzed by amylase.

Feed Quality of Winter and Summer Cereals

Quality tests show that winter triticale/wheat hay, pearl millet hay, and sweet sorghum hay, ensiled with and without an enzyme additive or untreated, have good relative feed values (91%–120%), but winter triticale/wheat straw has very low relative feed values (52%–61%) (Table 6). Protein content ranged from 8% to 19% for hays and from 2% to 6% for straws. Pearl millet and sweet sorghum hay have relatively greater NO_3 contents than winter triticale/wheat hay, which might cause potential toxicity to livestock.

CONCLUSIONS

Biofuel feedstock production may be integrated with existing livestock production systems in Montana and multi-product crops evaluated in this study can be used for biofuel feedstock and livestock feed. Double cropping winter cereal forage with warm season cereal crops increases total biomass and sugar yield per hectare, but suitable microorganisms are needed for more efficient fermentation of different forms of sugars to ethanol. While chemical pretreatment is suitable for triticale and wheat straw, the enzyme-assisted ensiling is effective for hay feedstocks.

Table 6. Feed quality of winter and summer cereal crops grown in central Montana in 2004 and 2005.

		Composition (%)					
Species	Treatment[x]	CP[y]	ADF	NDF	TDN	RFV	NO_3
Pearl millet hay	UT	19.3a[z]	31.5a	60.2a	64.7a	97.2a	2.7a
	EWE	15.6b	28.0a	45.8b	61.1a	120.0a	1.7a
	EWOE	14.5b	30.5a	54.6ab	59.9a	97.3a	1.8a
Sweet sorghum hay	UT	14.0a	33.2a	60.3a	64.7a	97.4b	1.3a
	EWE	11.0a	29.0a	47.2b	62.1a	118.0a	0.8a
	EWOE	11.7a	29.8a	52.5ab	62.3a	106.5b	0.9a
Winter triticale/wheat hay	UT	8.0b	37.7a	62.8a	56.0b	83.5b	0.2a
	EWE	13.6a	35.6a	59.4b	62.0a	95.8a	0.2a
	EWOE	12.0a	34.9a	62.8a	62.8a	91.4a	0.4a
Winter triticale/wheat straw	UT	2.1a	54.2a	83.6a	40.8a	51.9b	0.0a
	EWE	4.0a	52.9a	80.8a	42.2a	54.9ab	0.0a
	EWOE	5.8a	50.0a	76.4a	45.5a	60.8a	0.0a
Sorghum × sudangrass	UT	13.1	33.4	60.9	64.5	96.0	1.1

[z]Values followed by the same letter within each species and material are not significant different according to LSD multiple range test at P=0.05.

[y]CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, TDN = total digestible nutrients, RFV = relative feed value, and NO_3 = nitrate content.

[x]UT = untreated, EWE = ensiled with an enzyme additive, and EWOE = ensiled without an enzyme additive.

REFERENCES

Chen, Y., R.R. Sharma-Shivappa, and C. Chen. 2007a. Ensiling agricultural residues for bioethanol production. Appl. Biochem. Biotech. (In press).

Chen, Y., R.R. Sharma-Shivappa, D. Keshwani, and C. Chen. 2007b. Potential of agricultural residues and hay for bioethanol production. Appl. Biochem. Biotech. (In press).

Chinn, M.S., S.E. Nokes, and H.J. Strobel. 2006. Screening of thermophilic anaerobic bacteria for solid substrate cultivation on lignocellulosic substrates. Biotechnol. Prog. 22:53–59.

Henderson, N. 1993. Sillage additives. Animal Feed Sci. Technol. 45:35–56.

Jung, H.J.G. and J.F.S. Lamb. 2004. Prediction of cell wall polysaccharide and lignin concentrations of alfalfa stems from detergent fiber analysis. Biomass Bioenergy 27:365–373.

Lueschen, W.E., D.H. Putnam, B.K. Kanne, and T.R. Hoverstad. 1991. Agronomic practices for production of ethanol from sweet sorghum. J. Prod. Agr. 4:619–625.

Linden, J.C., L.L. Henk, V.G. Murphy, D.H. Smith, B.C. Gabrielsen, R.P. Tengerdy, and L. Czako. 1987. Preservation of potential fermentables in sweet sorghum by ensiling. Biotechnol. Bioeng. 30:860–867.

Lynd, L.R. 1996. Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. Ann. Rev. Energ. Env. 21:403–465.

Miller, G.L. 1959. Use of Dinitrosalicylic Acid Reagent for determination of reducing sugar. Anal. Chem. 31:426–428.

Montana Department of Agriculture. 2006. Montana Agricultural Statistics 2006. USDA-NASS Montana Field Office, Helena, MT.

Palmarola-Adrados, B., P. Choteborska, M. Galbe, and G. Zacchi. 2005. Ethanol production from non-starch carbohydrates of wheat bran. Bioresour. Technol. 96:843–850.

Renewable Fuels Association (RFA). 2007. Ethanol Industry Statistics: Historic U.S. fuel ethanol production. www.ethanolrfa.org/industry/statistics/ Accessed on January 18, 2007.

Richard, T.L., S. Proulx, K.J. Moore, and S. Shouse. 2001. Ensilage technology for biomass pre-treatment and storage. 2001 ASAE Annu. Int. Meeting, Sacramento, CA. July 30–Aug. 1, 2001.

Schmidt, J., J. Sipocz, I. Kaszas, G. Szakacs, A. Gyepes, and R.P. Tengerdy. 1997. Preservation of sugar content in ensiled sweet sorghum. Bioresour. Technol. 60:9–13.

Wang, M., C. Saricks, and D. Santini. 1999. Effects of fuel ethanol use on fuel-cycle energy and greenhouse gas emission. Research Repost. Center for Transportation Research, Energy Systems Division, Argonne National Laboratory, Argonne, IL.

Weinberg, Z.G. and G. Ashbell. 2003. Engineering aspects of ensiling. Biochem. Eng. J. 13:181–188.

Wilkinson, J.M., K.K. Bolsen, and C.J. Lin. 2003. Silage science and technology. p. 1–30. In: D.R. Boxton, R.E. Muck, and J.H. Harrison (eds.), Agronomy Monograph 42. ASA-CSSA-SSSA, Madison, WI.

Wyman, C.E. 1999. Biomass ethanol: Technical progress, opportunities, and commercial challenges. Ann. Rev. Energ. Env. 24:189–226.